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Research Article

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Spectrochemical Analysis of Sodium, Potassium, Calcium, Magnesium, Copper, and Zinc in Normal Human Erythrocytes *

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Although the mineral composition of the erythrocytes has been the subject of investigation for nearly half a century, the exact concentration of many of the elements in the cell has not been firmly established, and the information that is available is widely scattered throughout the literature. In most reports the composition of the cell is expressed per unit weight, per unit volume of packed cells, or per unit of cell water; there are only a few instances in which the average amount of element per cell has been reported (1–3).

The average sodium concentration in centrifuged erythrocytes has been reported to be from as low as 168 μg per g (4) to as high as 443 μg per g (5), and several textbooks (6–8) give a value of approximately 400 μg per g, which is not in accord with the results of recent investigation (9–13). In a few studies (9–13) correction for sodium in trapped plasma has been taken into consideration, but in others (14–21) it has been neglected. The removal of trapped plasma by washing erythrocytes has been recommended; however, the effect of this procedure on the chemical composition of the cell has not been extensively studied. In 1939 Streef (15) reported that the sodium concentration of erythrocytes declined when the cells were washed with isotonic salt or sugar solution, but recently Freeman and Spirtes (4) advocated washing erythrocytes with magnesium chloride for sodium analysis. Even though trapped plasma is not an important consideration in the determination of erythrocyte potassium, different laboratories have reported normal mean values that vary by as much as 2,400 μg per g (18, 22). The average erythrocyte magnesium concentration is given in a recent textbook (6) as 30 μg per ml, but the majority of the values reported in the literature are approximately twice this level. There still appears to be doubt about the presence of calcium in the erythrocyte (6, 7) although important physiological functions have been ascribed to it (23–25). The concentration of calcium has been variously reported by different workers to range from 0 to as high as 18 μg per g of cells (15, 16, 20, 26, 27).

The purpose of this report is to describe a method employing an emission spectrophotometric technique (28) for the analysis of sodium, potassium, calcium, magnesium, copper, and zinc in erythrocytes, to present normal values in a group of 50 control subjects, and to compare the results with normal values in the literature. In addition a study has been made of the effect of sodium EDTA and washing procedures on the chemical composition of the erythrocyte, and two different methods of correcting for trapped intercellular plasma have been evaluated.

Methods

Reagents and chemicals

The preparation of ultrapure water, purified heparin, and concentrated hydrochloric acid has been reported previously (28, 29). Spectrographically standardized pure salts [1] were used in the preparation of standard solutions, and other chemicals were certified reagent grade.[2] Trapped plasma measurements were made with RISA,[3] which was stored in contact with Amberlite IRA 400 enclosed in a dialysis bag (30). A choline-Tris buffer used for washing erythrocytes contained 0.108 M choline chloride and 0.05 M trihydroxymethylaminomethane. The pH was adjusted to 7.35 with HCl, and

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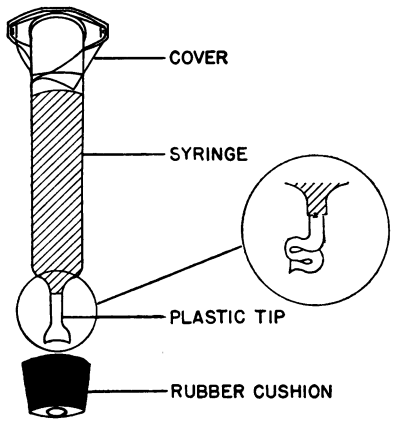

FIG. 1. PLASTIC SYRINGE USED FOR COLLECTION OF BLOOD AND THE SEPARATION OF PLASMA AND CELLULAR CONSTITUENTS SHOWN WITH THE METHOD FOR SECURING PLASTIC TIP ON NOZZLE BEFORE CENTRIFUGATION.

the osmolarity of the solution was 285 mOsm per L. A solution containing 285 mOsm per L of magnesium chloride used in washing erythrocytes was prepared from the spectrographically pure salt and the pH adjusted to 7.35 just before use. Five per cent sodium EDTA was purified by extraction with dithizon (31) and the pH adjusted to 7.35 with NaOH.

Cleaning of apparatus

The procedure for cleaning glass and polyethylene ware, plastic syringes, and platinum crucibles has been described elsewhere (28). Stainless steel needles were cleaned in dilute detergent, rinsed with ultrapure water, placed in pyrex glass tubes, and sterilized by heat at 103° C for 12 to 18 hours. Micropipettes were cleaned with detergent, soaked in an equal mixture of concentrated nitric and sulfuric acids, rinsed with ultrapure water, and dried by drawing acetone and then ether through them. Polyethylene adapters and tubing were cleaned with a dilute solution of detergent, rinsed with ultrapure water, soaked in 20% hydrochloric acid for 24 hours, rinsed with ultrapure water, and dried before use.

Sample collection and distribution

With a 30-ml plastic syringe [4] fitted with a stainless steel needle,[5] blood was taken from patients after an overnight fast. One-tenth mg of purified heparin was placed in the syringe before the blood was drawn. In certain studies trapped plasma was measured by adding about 10 μc of RISA to the blood before centrifugation. Three ml of blood was removed from the syringe for the determination of hemoglobin, hematocrit, red blood count, and blood smear. The red blood cells were separated from the blood plasma and other cellular constituents by centrifugation of the blood in the syringe at 2,000 $\times$ *g* at 4° C for 60 minutes. Before centrifugation the plunger was removed, the open end of the syringe was sealed with a plastic cover, a plastic tip [6] was secured on the nozzle with plastic tape,[7] and the syringe was supported by a rubber cushion (Figure 1). After centrifugation the plasma was removed with a polyethylene pipette and the erythrocytes were expressed from the base of the syringe by reinserting the plunger and attaching polyethylene tubing [8] to the nozzle of the syringe. Samples of the cells were then dispensed as follows: *1)* ½ g into a 5-ml volumetric flask for trapped plasma determination, *2)* 1 g into each of two platinum crucibles for analysis of calcium, magnesium, copper, and zinc, *3)* ½ g into a 5-ml volumetric flask for trapped plasma determinations, *4)* 1 g into a 50-ml Erlenmeyer flask for analysis of erythrocyte water, *5)* 100 mg into each of two 25-ml volumetric flasks for erythrocyte counts, *6)* 1 g into each of two 40-ml pyrex glass centrifuge tubes for flame spectrophotometric analysis of sodium and potassium, *7)* 10 mg into each of two aluminum boats for nitrogen analysis, and *8)* ½ g into a 5-ml volumetric flask for trapped plasma determination.

Analysis of plasma

Plasma water. One g of plasma was weighed into an Erlenmeyer flask and dried at 105° C for 24 hours. The water content was calculated from the decrease in weight of the sample.

Plasma sodium and potassium. A flame spectrophotometric technique (32) employing a Beckman DU spectrophotometer with flame attachment was used. The mean and standard deviation of measurements made on a single sample of plasma at intervals over a period of 6 months were for sodium 143 $\pm$ 2.0 mEq per L and for potassium 4.88 $\pm$ 0.23 mEq per L.

Plasma calcium. A flame spectrophotometric technique described by MacIntyre (33) was used with minor modifications. Trichloroacetic acid was substituted for perchloric acid, and both the anion and cation concentrations of the standard solutions closely approximated plasma. The mean and standard deviation of measurements made on a single sample of plasma at intervals over a 6-month period were 5.35 $\pm$ 0.09 mEq per L.

Plasma magnesium. The samples were analyzed by an emission spectrophotometric technique employing a vacuum cup electrode assembly in a Jarrell Ash, 3.4-meter direct-reading spectrograph (34). The mean and standard deviation of measurements made on a single sample of plasma over a period of 6 months were 1.66 $\pm$ 0.08 mEq per L.

Quantitative analysis of erythrocytes

Correction for trapped intercellular plasma. Cell counts, calcium, potassium, sodium, and water estima-

[4] J. F. Hartz, Toronto, Ontario.

[5] Becton, Dickinson, Toronto, Ontario.

[6] Stip, MacBrick, Cambridge, Mass.

[7] Curad tape, Kendall Co., Toronto, Ontario.

[8] Scalp vein infusion set, Abbott Laboratories, Montreal, Quebec.

tions on the centrifuged erythrocytes were corrected for trapped intercellular plasma. In earlier studies a composite calibration curve prepared from measurements of trapped plasma by an Evans blue dye technique in 20 control subjects was used. Trapped plasma varied in a linear manner from 1.5% at the bottom of the centrifuged cells to 4% at the top of the packed cell mass, and the relative standard deviation of the results from the mean curve was 18%. In later studies trapped plasma was measured in each syringe of packed cells by adding 10 μc of RISA to the blood before centrifugation (13). The radioactivity in samples of plasma and hemolysates of packed cells was counted in a well-type scintillation counter. Percentage of trapped plasma was calculated and a calibration curve constructed for each syringe of blood. Correction of measurements on packed cells for trapped plasma was made as follows: $C = [Cc - (Tp \times Cp)]/[100 - Tp]$, where C = constituent per microgram of cells; Cc = constituent per microgram of centrifuged cells; Cp = constituent per microgram of plasma, and Tp = trapped plasma (per cent).

Analysis of erythrocyte calcium, magnesium, copper, and zinc. The preparation of samples and their analysis by emission spectrophotometry have been described elsewhere (28, 35).

Analysis of erythrocyte sodium and potassium. One g of packed cells weighed into each of two 40-ml centrifuge tubes was hemolyzed by the addition of 20 ml of ultrapure water. Four ml of trichloroacetic acid was added to each sample, and the tubes were centrifuged at 1,200 *g* for 15 minutes. For potassium estimations the supernatant fluid was diluted 1:5 with water. The emission intensity of the samples and of standards prepared from stock solutions of NaCl and KCl were measured in a Beckman DU spectrophotometer with a flame attachment. The reproducibility of the method estimated from the repetitive analysis of a single sample over a period of 6 months was 9.69 ± 0.42 μmoles per g for sodium and 80.87 ± 3.64 μmoles per g for potassium. Both the sodium and potassium concentration of the packed cells was corrected for trapped intercellular plasma.

Erythrocyte water. One g of packed cells weighed into an Erlenmeyer flask was dried at 105° C for 24 hours. The water content calculated from the dried weight was corrected for water in trapped intercellular plasma. The mean and standard deviation of the technique determined from ten measurements on a pooled sample of red blood cells were 679.3 ± 2.2 mg per g.

Erythrocyte hemoglobin. The hemoglobin concentration of whole blood was determined in duplicate by the cyanmethemoglobin method (36), and the optical density was measured in a Beckman model B spectrophotometer. The mean value and standard deviation of hemoglobin levels in 25 female and 25 male control subjects were 13.7 ± 0.9 g per 100 ml and 15.1 ± 1.0 g per 100 ml, respectively. The reproducibility of the method was evaluated from duplicate estimations on 12 randomly selected samples of blood; the mean and standard deviation of the results were 11.9 ± 0.4 g per 100 ml. Erythrocyte counts were measured in quadruplicate with a conventional electronic cell counter. The mean and standard deviation of results in 25 female and 25 male subjects were $4.53 \pm 0.31 \times 10^6$ cells per mm^3 and $4.93 \pm 0.37 \times 10^6$ cells per mm^3, respectively. The mean and standard deviation determined from duplicate estimations on 12 randomly selected samples of blood were $3.93 \pm 0.04 \times 10^6$ cells per mm^3. The mean erythrocyte hemoglobin was determined by dividing the hemoglobin concentration by the erythrocyte count.

Erythrocyte volume. The hematocrit of whole blood was measured in duplicate by a microhematocrit method (37). The mean and standard deviation in 25 female and 25 male control subjects were $40.4 \pm 1.5\%$ and $44.9 \pm 3.1\%$, respectively. The mean and standard deviation determined from duplicate estimations of 12 randomly selected samples of blood were $36.3 \pm 0.5\%$. The mean erythrocyte volume was obtained by dividing the hematocrit by the erythrocyte count.

Erythrocytes per gram of packed cells. Approximately 0.1 g of packed cells weighed into each of two 25-ml volumetric flasks was diluted in 0.9% sodium chloride containing per L 5 ml of 36% formaldehyde. Twenty μl of this suspension was diluted in 20 ml of 0.9% sodium chloride, and red blood counts were made in duplicate with the electronic cell counter. The result was corrected for trapped plasma.

Erythrocyte nitrogen. Ten mg of packed cells was weighed into each of two aluminum boats. The nitrogen concentration was measured with a Coleman nitrogen analyzer (38). The mean and standard deviation of the technique determined from ten duplicate determinations were 52.0 ± 1.9 $\mu g \times 10^3$ per g.

Expression of results. Determination of the amount of a particular erythrocyte constituent per cell, per cubic micron of cell, per microliter of cellular water, per micromole of hemoglobin, or per micromole of cellular nitrogen was made with an electronic digital computer. The calculations were as follows: element per cell (micromoles) = (microgram of element per gram packed cells/no. of erythrocytes per gram of packed cells) $\times$ (1/mol wt); element per cubic micron of cell (micromoles) = micromoles of element per cell/mean cell volume; element per microliter of cell water (micromoles) = (micromoles of element per gram of packed cells $\times 10^3$)/micrograms of water per gram of packed cells; element per micromole of hemoglobin (micromoles) = micromoles of element per cell/micromoles of hemoglobin per cell; element per micromole of cell nitrogen (micromoles) = micromoles of element per gram of packed cells/micromoles of nitrogen per gram of packed cells.

Selection of control subjects

Normal values were established for 25 men and 25 women selected from both medical personnel and patients who on enquiry were free from organic disease and who were found on investigation to have a normal blood smear and a hemoglobin, hematocrit, red blood count, and erythrocyte sedimentation rate within the normal range (6).

TABLE I

Comparison of the effect of heparin and Na_4 EDTA on the chemical composition of normal erythrocytes

Element	Amount of constituent per cell (mean value ± SD) Heparin	Na_4 EDTA	*t* value*
Water, $\mu l \times 10^{-9}$	73.3 ± 6.5	74.3 ± 6.8	
Sodium, $\mu moles \times 10^{-9}$	0.930 ± 0.15	0.990 ± 0.20	
Potassium, $\mu moles \times 10^{-9}$	10.3 ± 0.64	10.1 ± 0.67	
Magnesium, $\mu moles \times 10^{-9}$	0.221 ± 0.083	0.215 ± 0.002	
Calcium, $\mu moles \times 10^{-12}$	3.38 ± 0.39	2.11 ± 0.75	13.7
Copper, $\mu moles \times 10^{-12}$	1.36 ± 0.18	1.43 ± 0.20	
Zinc, $\mu moles \times 10^{-12}$	16.3 ± 2.4	16.8 ± 2.9	

* Given where $p < 0.05$.

Their ages varied between 16 and 75 years. In this group correction of certain results for trapped plasma was made from a composite calibration curve. Normal values were also established in 16 control subjects in whom erythrocyte values were corrected from individual calibration curves prepared by measuring trapped plasma in each sample with RISA.

Effect of anticoagulant on the chemical composition of erythrocytes

Two samples of blood were taken from each of eight control subjects. One-tenth mg of purified heparin was used as the anticoagulant for one sample, and 1 ml of 5% Na_4 EDTA was used for the other. The mineral composition of the erythrocytes was determined as described previously.

Effect of washing erythrocytes on their chemical composition

Two samples of blood were taken from each of eight control subjects. One sample from each subject was analyzed as described previously; the other was centrifuged at 2,000 × *g* at 4° C for 20 minutes, and the plasma and buffy coat were removed. The erythrocytes were suspended in a cold solution containing 285 mOsm per L of magnesium chloride, and centrifugation was repeated. The supernatant was discarded, the cells were resuspended in magnesium chloride, and 10 μc of RISA was added for the determination of trapped buffer. The blood was centrifuged at 2,000 × *g* at 4° C for 60 minutes, and the packed cells were analyzed as described previously. A similar experiment was performed with choline-Tris buffer instead of magnesium chloride.

Results

The use of sodium EDTA rather than heparin as an anticoagulant led to significantly lower values for erythrocyte calcium, but the levels of the other minerals in the cell were unaffected (Table I).

The effect of washing erythrocytes with magnesium chloride and with choline-Tris buffer on their chemical composition is shown in Table II. Sodium per cell was moderately decreased, and calcium per cell was markedly decreased by the washing process.

The effects of correcting sodium and calcium concentrations in packed cells for trapped intercellular plasma, determined from a composite calibration curve and from individual curves prepared by measuring trapped plasma in each sample with RISA, are shown in Table III. The mean concentration of sodium in the erythrocytes of the two groups is similar, but the standard deviation and range of results are much smaller in the group in which trapped plasma corrections were made from individual calibration curves. The average amount of erythrocyte calcium is lower in the

TABLE II

Effect of washing erythrocytes on their chemical composition

Element	Amount of constituent per cell (mean value ± SD) Effect of choline-Tris buffer: Unwashed cells	Washed cells	*t* value*	Effect of magnesium chloride: Unwashed cells	Washed cells	*t* value*
Water, $\mu l \times 10^{-9}$	73.3 ± 6.5	75.7 ± 5.2		70.6 ± 6.8	71.6 ± 6.9	
Sodium, $\mu moles \times 10^{-9}$	0.930 ± 0.15	0.760 ± 0.18	6.3	0.900 ± 0.15	0.750 ± 0.75	5.9
Potassium, $\mu moles \times 10^{-9}$	10.3 ± 0.64	10.2 ± 0.73		9.72 ± 1.2	9.40 ± 1.1	
Magnesium, $\mu moles \times 10^{-9}$	0.221 ± 0.083	0.236 ± 0.014		0.241 ± 0.091	0.277 ± 0.080	
Calcium, $\mu moles \times 10^{-12}$	3.38 ± 0.39	1.72 ± 0.54	23.1	3.52 ± 0.32	2.10 ± 0.92	12.
Copper, $\mu moles \times 10^{-12}$	1.36 ± 0.18	1.46 ± 0.19		1.44 ± 0.17	1.52 ± 0.28	
Zinc, $\mu moles \times 10^{-12}$	16.3 ± 2.4	17.1 ± 2.9		16.2 ± 1.0	16.2 ± 1.4	

* Given where $p < 0.05$.

TABLE III

Effect of trapped plasma correction from composite calibration curve and from individual curves prepared from measurements with I^{131}-albumin on value of erythrocyte Na and Ca in control subjects

Element	Trapped plasma correction			
	Calibration curve		I^{131}-albumin	
	Mean ±SD	Range	Mean ±SD	Range
Sodium				
μmoles per g	8.79 ± 1.3	5.60 – 11.2	8.84 ± 0.91	7.39 – 10.4
μmoles × 10^{-9} per cell	0.934 ± 0.14	0.643 – 1.30	0.914 ± 0.10	0.778 – 1.09
μmoles × 10^{-12} per μ^3	10.3 ± 1.7	6.56 – 13.6	10.0 ± 0.94	8.39 – 11.8
μmoles × 10^{-3} per μl H_2O	12.9 ± 2.0	8.21 – 16.8	13.2 ± 1.37	11.2 – 15.4
Calcium				
μmoles × 10^{-3} per g	59.4 ± 17	25.0 – 67.5	35.9 ± 9.3	21.0 – 50.2
μmoles × 10^{-12} per cell	6.32 ± 1.8	2.50 – 10.0	3.72 ± 0.99	2.0 – 5.25
μmoles × 10^{-15} per μ^3	69.7 ± 20	28.5 – 113	40.9 ± 11	20.4 – 56.5
μmoles × 10^{-6} per μl H_2O	87.3 ± 25	35.0 – 133	53.8 ± 14.3	30.5 – 77.0

group in which RISA was used to correct for trapped plasma, and the deviation of the results from the mean is smaller.

The mean and standard deviation of erythrocyte sodium, potassium, magnesium, calcium, copper, zinc, water, hemoglobin, and nitrogen in 50 control subjects are given in Table IV. In this group correction for trapped intercellular plasma was made with a composite calibration curve. No difference in the composition of erythrocytes was found between males and females, nor was any relation to age noted. The frequency distribution of each of the results about the mean value is shown in Figure 2. It will be seen that apart from nitrogen they were distributed in a normal manner.

The concentration of total cations per liter of erythrocyte water was slightly lower than the total cation concentration per microliter of plasma water (Table V).

No significant correlation was found between the

TABLE IV

Chemical composition of erythrocytes in control subjects (mean value ± SD)

	μg per g	*μmoles per g*	*μmoles × 10^{-9} per cell*	*μmoles × 10^{-12} per μ^3 of cell*	*μmoles × 10^{-3} per μl H_2O*	*μmoles per μmole hemoglobin*	*μmoles × 10^{-3} per μmole nitrogen*
Sodium	202 ± 31	8.79 ± 1.3	0.934 ± 0.14	10.3 ± 1.7	12.9 ± 2.0	2.04 ± 0.35	4.45 ± 0.68
Potassium	3,623 ± 104	92.67 ± 2.7	9.87 ± 0.56	108.7 ± 5.7	136 ± 4.0	21.46 ± 1.3	47.0 ± 2.23
Magnesium	57.0 ± 8.4	2.34 ± 0.35	0.250 ± 0.037	2.75 ± 0.42	3.44 ± 0.49	0.542 ± 0.078	1.19 ± 0.18
Zinc	10.9 ± 1.9	0.167 ± 0.029	0.0178 ± 0.0032	0.195 ± 0.033	0.245 ± 0.040	0.0386 ± 0.0068	0.0846 ± 0.015
	μg per g	*μmoles × 10^{-3} per g*	*μmoles × 10^{-12} per cell*	*μmoles × 10^{-15} per μ^3 of cell*	*μmoles × 10^{-6} per μl H_2O*	*μmoles × 10^{-3} per μmole hemoglobin*	*μmoles × 10^{-6} per μmole nitrogen*
Calcium	2.38 ± 0.68	59.4 ± 17	6.32 ± 1.8	69.7 ± 20	87.3 ± 25	13.8 ± 4.2	30.1 ± 8.4
Copper	0.95 ± 0.13	14.9 ± 2.0	1.59 ± 0.23	17.5 ± 2.7	21.9 ± 30	3.45 ± 0.49	7.56 ± 1.1
	μg × 10^3 per g	*μmoles per g*	*μmoles × 10^{-9} per cell*	*μmoles × 10^{-12} per μ^3 of cell*			
Hemoglobin	289 ± 19	4.33 ± 0.23	0.461 ± 0.030	5.07 ± 0.27	Mean cell volume 91.0 ± 5.2 μ^3		
	μg × 10^3 per g	*μmoles × 10^3 per g*	*μmoles × 10^{-9} per cell*	*μmoles × 10^{-12} per μ^3 of cell*			
Nitrogen	55.3 ± 1.8	1.97 ± 0.64	210 ± 11	2,315 ± 110			
	μg × 10^3 per g	*μl per g*	*μl × 10^{-9} per cell*	*μl × 10^{-12} per μ^3 of cell*			
Water	681.3 ± 13.6	681.3 ± 13.6	72.5 ± 3.6	799.0 ± 42			

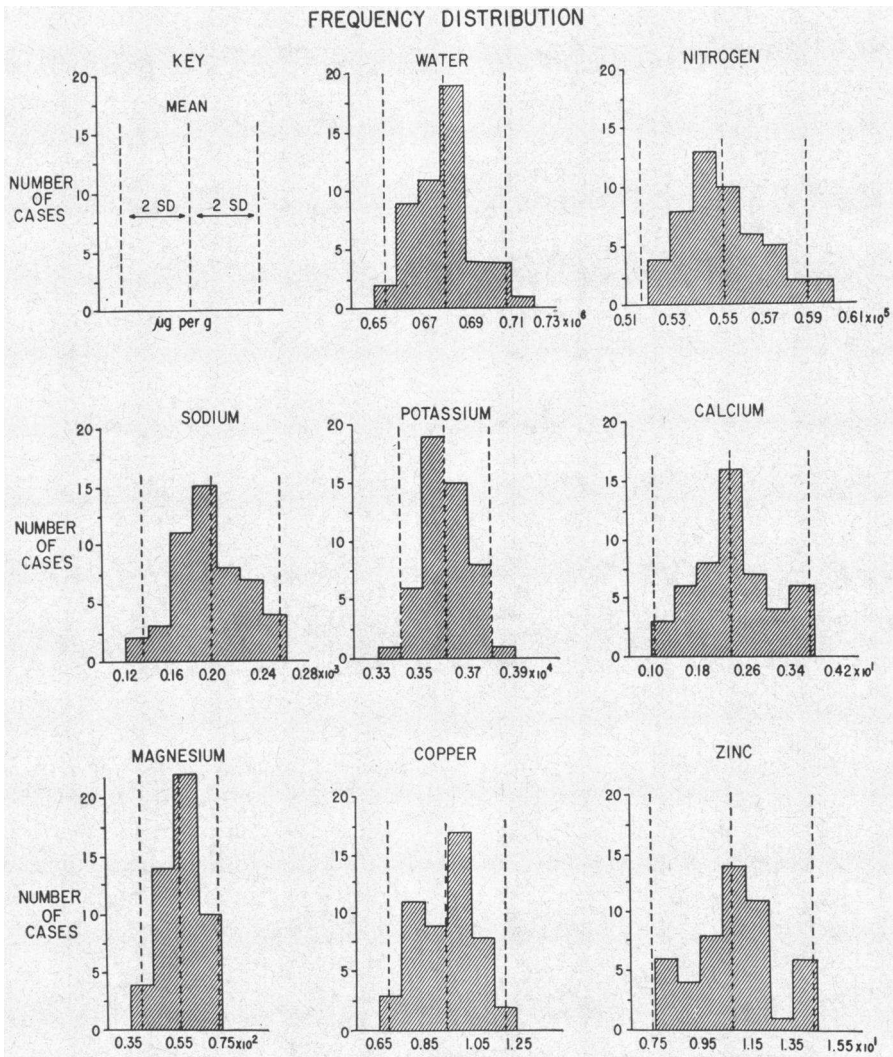

FIG. 2. FREQUENCY DISTRIBUTION OF ERYTHROCYTE CONSTITUENTS.

plasma concentration of sodium, potassium, calcium, and magnesium and any of the mineral constituents in the erythrocyte. The mean volume of the erythrocytes was significantly correlated ($p < 0.01$) with hemoglobin per cell ($r = 0.640$), nitrogen per cell ($r = 0.628$), water per cell ($r = 0.512$), potassium per cell ($r = 0.576$), and sodium per cell ($r = 0.591$). No significant correlation was found between mean cell volume and the amount of calcium, magnesium, copper, or zinc per cell.

Discussion

The relatively high concentration of sodium and calcium in plasma makes correction for these elements in trapped intercellular plasma mandatory when analyses are performed on packed cells. Removal of trapped plasma by washing the cells with magnesium chloride as suggested by Freeman and Spirtes (4) or with choline-Tris buffer (39) is unsatisfactory because both sodium and calcium are eluted from the cells (Table II). Determination of erythrocyte sodium from the difference between the sodium concentration of plasma and of whole blood is inaccurate, and normal values reported with this method are unusually high (Table VI). Correction for trapped plasma from a composite calibration curve is not completely satisfactory. Although the speed and duration of centrifugation of the blood are kept constant and samples

TABLE V

Cation composition of plasma and erythrocytes in normal subjects

Element	Average concentration and SD of element	
	Plasma	Erythrocyte
	mEq/L water	
Sodium	150.10 ± 3.2	12.91 ± 1.9
Potassium	5.47 ± 0.42	136.08 ± 23
Calcium	5.83 ± 0.61	0.18 ± 0.05
Magnesium	1.85 ± 0.23	6.88 ± 0.98
Total	163.25	156.05

are taken from the same level in the packed cell mass, the amount of trapped plasma shows considerable variation among subjects. This undoubtedly contributed to the relatively wide range of results for erythrocyte sodium and calcium given in Table IV for control subjects. When correction for the amount of trapped plasma in each individual blood sample was made with RISA, the range of concentration of these elements in the erythrocytes was much narrower (Table III). This finding is in accord with the results of a recent study by Czaczkes, Ullman, Ullman, and Bar-Kochba (13).

Studies made of possible analytical errors arising from the inclusion of a higher percentage of young cells in samples taken from the upper part of the packed cell mass showed that although there was a higher proportion of young cells in the upper part (2.6%), the difference between the uppermost and lowermost layers was small (1.2%). No appreciable difference was found in the concentration of erythrocyte sodium, potassium, calcium, magnesium, copper, zinc, nitrogen, and water or in cell volume at different levels of the packed cell mass providing corrections were made for trapped intercellular plasma.

In the present study the average hemoglobin per cell and per cubic micron of cell in the control subjects is similar to published values (6, 10, 14). The mean value for erythrocyte nitrogen is higher than the results reported by Dennes, Tupper, and Wormall (41) and McCance and Widdowson (10), and a negative skewness is present in the histogram of the results (Figure 2). This is probably due to wide variations in instrument blank values that were encountered occasionally with the Coleman nitrogen analyzer in our early studies. In this respect our experience has been similar to that of Flokstra and Nodolski (38). The trouble was eventually corrected by replacing the syringe vent valve and the components in the combustion train between the post heater tube and the nitrometer. The mean nitrogen value in subsequent control subjects was 53.3×10^3 μg per g, and the results were distributed in a normal manner about the mean.

The average value for erythrocyte water, 681.3

TABLE VI

Comparison of erythrocyte sodium values in control subjects with results in literature

No. of cases	Mean	Range	Method	Trapped plasma correction	Reference
	μg/g	μg/g			
50	202	129–257	Direct	Calibration curve	Present study
16	203	170–240	Direct	I^{131}-albumin	Present study
12	209	167–251	Direct	Calibration curve	Selwyn and Dacie (9)
5	215		Direct	Calibration curve	McCance and Widdowson (10)
14	194	145–243*	Direct	Calibration curve	Solomon (11)
42	238	145–336	Direct and indirect	From duplicate samples	Choremis, Economou-Mavrou, and Tsenghi (12)
20	179	156–214	Direct	I^{131}-albumin	Czaczkes, Ullman, Ullman, and Bar-Kochba (13)
12	168	105–231*	Direct	Washed with $MgCl_2$	Freeman and Spirtes (4)
160	303	82–562	Direct	None	Others (15–21)
65	366	161–552	Indirect		Hald and Eisenman (27); Nichols and Nichols (40); Kessler, Levy, and Allen (22)

* Range based on ± standard deviation.

TABLE VII

Comparison of erythrocyte potassium values in control subjects with results in literature

No. of cases	Mean	Range	Reference
	μg/g	μg/g	
50	3,623	3,377–3,839	Present study
6	3,782	3,604–3,925	Maizels (14)
25	3,464	2,980–3,821	Streef (15)
8	3,366	3,250–3,540	Hald (5)
21	3,358	3,002–3,714*	Solomon (11)
93	3,512	3,313–4,106	Lans, Stein, and Meyer (45)
14	3,371	3,115–3,627*	Hutt (42)
21	3,729	3,271–4,187*	Nichols and Nichols (40)
22	3,500	3,342–3,695	Keitel (17)
35	3,401	3,133–3,667*	Dowbin and Holley (19)
12	3,752	3,540–4,036	Selywn and Dacie (9)
20	3,627	3,198–3,939	Czaczkes and associates (13)
47	3,349	2,895–3,803*	Videbaek and Ackermann (46)
30	3,383	3,140–3,782	Gerchikova (21)
103	<3,300	2,407–3,646	Freeman and Spirtes (4); Snyder and Katzenelbogen (16); Hald and Eisenman (27); Singer, Hoff, Fisch, and DeGraff (47); and Riecker (18)
84	>3,800	3,176–5,694	Kramer and Tisdall (26), Hoffman and Jacobs (48), Kessler and associates (22), Choremis and associates (12), and McCance and Widdowson (10)

* Range based on ± 2 SD.

$\times 10^3$ μg per g, is in close agreement with previous experimental values (10, 12–14, 17, 18, 22, 40, 42). The value of 33.15 g per 100 ml for erythrocyte water given by Wintrobe (6) and reproduced in a table by Harris (8) is a typographical error that should read 63.15 g per 100 ml (43).

The mean value for erythrocyte sodium per gram of packed cells is similar to the level found by Solomon (11) and Selwyn and Dacie (9), but it is 13% higher than the results obtained by Czaczkes and co-workers (13), 6% lower than the mean value found by McCance and Widdowson, and 18% less than the value given by Choremis and associates (12) in children (Table VI). The discrepancy in results is probably due to minor differences in the composition of standards, in excitation conditions, or in the instruments used in the analysis. The low value of erythrocyte sodium reported by Freeman and Spirtes (4) can be accounted for by their use of magnesium chloride to wash erythrocytes free from trapped intercellular plasma. The high mean values for erythrocyte sodium reported by many workers can be attributed either to a failure to correct for trapped intercellular plasma (14–21) or to the inaccuracy of the indirect method of analysis in which erythrocyte sodium is calculated from the difference in measurements made on whole blood and serum (22, 27, 40). The latter explains the impression that the average single erythrocyte contains 32×10^{-9} μg of sodium (1, 44), which is considerably higher than the value of 22×10^{-9} μg obtained when correction is made for trapped intercellular plasma (Table III).

The mean and range of potassium per gram of packed cells in our control subjects are in accord with the results of most studies, but there are mean values, even in the recent literature, that are much higher and much lower (Table VII).

Our findings lend support to the idea that calcium is a constituent of the erythrocyte. The mean value of 2.38 μg per g or 0.594 μmoles per g of cells is 40% lower than the levels found by Bolingbroke and Maizels (25) in a single sample of washed cells, and it is different from other values reported in the literature, many of which are erroneously high because no correction has been made for calcium in trapped plasma (15, 16, 20, 26, 27). The large deviation of the calcium results from the mean (Table IV) is due to differences in the amount of trapped plasma in the packed cells that have not been taken into account when trapped plasma correction was made from a composite calibration curve. Small changes in trapped plasma produce relatively large changes in the results of erythrocyte calcium because there

is about 50 times more calcium in plasma than in the red blood cells. When trapped plasma correction is made from individual calibration curves determined with RISA, the deviation of results from the mean is much smaller, and the mean level in normal subjects is less than the level obtained with a composite calibration curve (Table III). The finding of a lower mean value for erythrocyte calcium when trapped plasma correction was made with RISA is due in part to greater accuracy of this method and in part to the fact that values for trapped plasma obtained with RISA are slightly higher than values obtained with Evans blue dye, the latter having been used to construct the composite calibration curve. In 18 samples of packed cells in which trapped plasma was measured simultaneously with Evans blue dye and with RISA, the mean level obtained with the former was 3.52% compared to 3.81% for the latter. Analysis of the difference between the two groups by the method of paired comparisons was statistically significant at $p < 0.001$. The reason for the slight discrepancy in the results of the two methods is not known. The ease with which calcium is removed from the erythrocyte by washing procedures and by Na_4 EDTA suggests that it either readily traverses the cell membrane or it is held in loose combination in the membrane or on the cell surface.

The mean and range of results for copper and magnesium per gram of cells are similar to those obtained by other workers (3, 49–52). The mean value for zinc per gram of erythrocytes compares favorably with results reported by Koch, Smith, Shimp, and Connor (50), Herring, Leavell, Paĭxao, and Yoe (49), and by Talbot and Ross (53), but it is lower than the mean value of 13.2 μg per g found by Vallee and Gibson (2) in control subjects. The average amount of zinc per cell is similar to the level reported by Fredricks, Tanaka, and Valentine (54) and Vallee and Gibson (2), but it is 20% greater than Dennes and co-workers (41) found in the erythrocyte by neutron activation analysis. The mean and standard deviation of the zinc per microgram of hemoglobin are the same as those reported by Zak, Nalbandian, Williams, and Cohen (55).

A degree of uncertainty still exists in the literature about the absolute level of total cation in the human erythrocyte. There are reports in which the total cation concentration per liter of cell water is greater than (5, 40, 44, 56, 57, 58) and others in which it is less than (14, 59) the total cation concentration of plasma. Some of the discrepancy can be attributed to the inaccuracy of methods in which erythrocyte values are derived from analyses performed on whole blood and serum (5, 40, 44, 56, 58) and to a failure to express the plasma cation concentration on the basis of plasma water (14). The findings in the present study support the concept that the total cation concentration per liter of erythrocyte water is less than the total cation concentration in the plasma (Table V). The amount of water freely available as solvent in the erythrocyte has been estimated to be approximately 93% (14, 60), and if the cation concentration obtained in the present study is corrected accordingly, the cation concentration of the intracellular fluid is higher than the level in the plasma, a finding that is in accord with the view that intracellular fluid must contain more cations than interstitial fluid for the fluids to be in osmotic balance (61). Accurate information is not available on the proportion of cation in the erythrocyte that is osmotically inactive, and it is not possible to assess the importance of this factor on the interpretation of the results.

Summary

1) Erythrocytes from 50 normal subjects were analyzed for sodium, potassium, calcium, magnesium, copper, and zinc. Normal values for each of these elements per gram of packed cells, per cell, per cubic micron of cell, per microliter of cell water, per micromole of hemoglobin, and per micromole of cell nitrogen are given. The average erythrocyte contained 0.93×10^{-9} μmoles of sodium, 9.87×10^{-9} μmoles of potassium, 0.25×10^{-9} μmoles of magnesium, 17.8×10^{-12} μmoles of zinc, 3.72×10^{-12} μmoles of calcium, and 1.59×10^{-12} μmoles of copper. The total cation concentration in erythrocyte water was 156.05 mEq per L compared to 163.25 mEq per L in plasma water.

2) In control subjects no correlation was found between the plasma concentration of sodium, potassium, calcium, or magnesium and the mineral constituents of the cell. The mean erythrocyte volume was correlated with hemoglobin per cell, water per cell, potassium per cell, and sodium per

cell. No correlation was found between mean cell volume and the amount of calcium, magnesium, copper, or zinc per cell.

3) Washing erythrocytes with either isotonic magnesium chloride or choline-Tris buffer eluted both sodium and calcium from the cells. The use of Na_4 EDTA as an anticoagulant removed calcium from the erythrocytes, but the levels of sodium, potassium, magnesium, copper, and zinc in the cell were not affected.

4) The comparatively high concentration of sodium and calcium in plasma makes correction for trapped intercellular plasma mandatory when analyses for these elements are made on packed cells. The range of erythrocyte sodium and both the mean value and range of erythrocyte calcium were smaller when correction for trapped plasma on each individual blood sample was made with RISA than the values obtained when a composite calibration curve prepared with Evans blue dye was used for trapped plasma correction.

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